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Response of Several Soybean Varieties to Co-inoculation with Rhizobium and Mycorrhiza Biofertilizers in Dryland of East Lombok, Indonesia

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Abstract*— *In Indonesia, soybean is the most important legume crop, which can develop Rhizobium and mycorrhizal symbiosis for better nutrition, especially in dryland areas. A trial for investigating responses of several varieties of soybean to co-inoculation with Rhizobium and mycorrhiza biofertilizers has been carried out in East Lombok, Indonesia, from August to October 2022. The Randomized Block Design was used to arrange the two treatment factors tested, i.e. biofertilizer application (B0= without biofertilizer; B1= Rhizobium inoculant; B2: co-inoculation of soybean with Rhizobium and mycorrhiza biofertilizers) and soybean varieties (V1= Detap, V2: Biosoy-2, V3= Dena-1). The trial was made in three blocks (replications). The variables included plant height, trifoliate number, root volume, and biomass yield and pod number as a yield potential. Results indicated that co-inoculation several varieties of soybean with Rhizobium and mycorrhiza biofertilizer increased yield potential of soybean in dryland as indicated by higher nodule number, trifoliate number, pod number and biomass yield of soybean, especially of Detap (V1) and Dena-1 (V3) varieties under co-inoculation treatment (B2), compared to inoculation with Rhizobium only (B1) or unioculated control (B0). Further studies on more varieties of soybean need to be conducted in different types of soil to find out the most responsive varieties to co-inoculation with Rhizobium and mycorrhiza biofertilizer to increase soybean productivity especially in dryland areas.

***Keywords*— Co-inoculation, Mycorrhizal fungi, Rhizobium, Soybean, Dryland.**

I. INTRODUCTION

Soybean (*Glycine max* (L) Merr) is the most important legume food crop in Indonesia, based on the total area harvested, which achieved 614,095 ha in 2015 (https://www.bps.go.id/indicator/53/21/1/luas-panen.html), with a total national production of 963,183 ton in 2015 (https://www.bps.go.id/indicator/53/23/1/produksi.html) and an average productivity of only 1,568 kg/ha (https://www.bps.go.id/indicator/53/22/1/produktivitas.html). Unfortunately this amount of total production could not meet the domestic need for soybean, so that soybean still has to be imported. In 2021 the total import of soybean was up to 2.5 million tons [1]. This means that soybean production in Indonesia is still very low compared with its consumption making the amount of soybean import of almost three times its production. Production of soybean can be increased by increasing its productivity and/or increasing the total area harvested through extension of soybean growing areas to dryland areas. In terms of soybean productivity, the average national productivity is still very low because some varieties of soybean under application of appropriate technology can achieve much higher productivity such as those described in the soybean description in which some varieties have a high productivity, such as Mutiara-1 and Dega-1 varieties with an average productivity of 4.1 and 3.82 ton/ha respectively (https://balitkabi.litbang.pertanian.go.id/).

Another way of increasing soybean production is by increasing the annual total of soybean harvested area, i.e. by increasing planting area and reducing the potential yield

loss during crop growth. Among the harvested soybean areas in Indonesia, 65% of the production areas are in the irrigated areas in which soybean is used as a rotation crop that is mostly planted in the dry season after harvest of irrigated rice. However, there are potential economic obstacles for increasing soybean harvested areas in the irrigated areas because some other crops are more profitable than soybean [2]. Another potential area for soybean production in Indonesia is dryland, with a total potential area of 4.29 millions ha [3], although there are various obstacles for getting a high yield of soybean from dryland areas due to the high variability of dryland conditions including low availability of nutrients and soil moisture, and low soil pH in some areas of dryland in Indonesia [4]. Even in an area of relatively fertile, from an experiment conducted during a dry season in south eastern Lombok, fertilization with N, P and K fertilizers was reported to show no significant effect of the NPK fertilization, in which the average soybean grain yield in Sengkol was only 1.48 ton/ha with NPK compared with 1.47 ton/ha without NPK fertilization [5]. These indicate that other components of production technologies are required for increasing soybean yield to be close to its potential yield.

In irrigated rice growing areas, the low average yield of soybean grown during the dry season in rotation with irrigated rice could be due to the low population of arbuscular mycorrhizal fungi (AMF) following flooded rice crops [6-7]. Wangiyana et al. [8] also reported that application of mycorrhiza biofertilizer on soybean direct-seeded following rice crop on vertisol soil was more significant in increasing grain yield of soybean grown following conventional rice than following SRI rice, which indicated a detrimental effect of flooded rice crop on the population of AMF in the rice field. In addition, to achieve relatively high yield, soybean requires very high amount of nitrogen during the seed-filling stage of growth, which is the highest among seed plants [9]. However, soybean plants are reported to be able to meet their nitrogen requirement up to 90% from nitrogen fixation resulted from their symbiosis with *Rhizobium* bacteria through formation of effective root nodules [4]. Therefore, establishment of symbiosis with *Rhizobium* is very important for high grain yield of soybean plants. In addition to symbiosis with *Rhizobium* bacteria, soybean plants can also establish symbiosis with AMF, resulting in a tripartite symbiosis [10-12]. In vertisol rice land, soybean grown following rice crop during a dry season showed significantly higher grain yield when inoculated with *Rhizobium* and mycorrhiza biofertilizer compared to inoculation with *Rhizobium* only followed with application of NPK fertilizer, which indicates the significant effect of co-inoculation with both types of the biofertilizer [13].

This study aimed to examine the effect of co-inoculation with *Rhizobium* and mycorrhiza biofertilizers on growth, nodulation, and biomass yield of several varieties of soybean in dryland area of East Lombok, Indonesia, with a sandy soil type.

II. MATERIALS AND METHODS

The field trial in this study was carried out on a farmer's dryland in Labuhan Lombok (East Lombok), Indonesia, with an Entisol soil type, from August to October 2022 (planting of soybean seeds of the three varieties was done on 20th of August, 2022). The Randomized Block Design was used to arrange the two treatment factors, i.e. biofertilizer application (B0= without biofertilizer; B1= *Rhizobium* inoculant; B2: co-inoculation of soybean with *Rhizobium* and mycorrhiza biofertilizers) and soybean varieties (V1= Detap, V2: Biosoy-2, V3= Dena-1). The trial was made in three blocks (replications). Therefore, there were 27 experimental units.

After harvesting previous crop (maize grown for seed production) and removing the plant debris, soil tillage was done by once plowing and harrowing, followed by formation of raised beds of 2.10 m length and 1.60 with a height of 15 cm from the base of the furrow surrounding the beds. Seeds of the three soybean varieties were dibbled under plant spacing of 30 cm between and 20 cm within rows by burying 3-4 seeds per planting hole. For the B1 and B2 treatments, seeds were coated with the *Rhizobium* inoculant. In the B2 treatment, the planting holes were first filled with Mycorrhiza biofertilizer of 7.5 gram per planting hole, which then covered with soil, and the coated seeds were placed above it, and then covered with soil. Seeds in the planting holes of treatment B0 and B1 were also covered with soil. The mycorrhiza biofertilizer used was those under the trade mark "Technofert" (a bio-fertilizer containing mixed species of AMF mixed in the zeolit growing media, produced by the BPPT biotechnology research institute, Serpong, Indonesia).

At 10 days after seeding (DAS) the soybean seeds, tinning was done by allowing to grow only 2 soybean plants per planting hole, which was followed with fertilization using Phonska fertilizer (NPK 15-15-15) by dibbling it at 7 cm depth and 7 cm next to young soybean plants at a dose of 200 kg/ha Phonska. Weeding was done manually on 21 and 42 DAS. Harvest of sample plants was done at 50 DAS to measure biomass yield, pod number and root volume (soybean plants have not achieved maturity when this paper was written). Other crop maintenance included insecticide sprays at 14 and 35 DAS to control several

insect pests such as larvae of seedling flies and plant-hoppers.

Plant growth variables (plant height and trifoliate number) were measured at 14, 28 and 42 DAS from eight clumps of soybean plant systematic random samples per plot, while root volume, root nodule number, and potential yield components (biomass yield and pod number) were measured from one clump of soybean plant harvested (uprooted) at 50 DAS (seed-filling stage), which was randomly selected from the eight clumps of sample plants per plot. Root volume was measured using measuring glass by immersing the root into the water in the measuring glass. For analyzing the data, CoStat for Windows ver. 6.303 was used for running ANOVA and Tukey's HSD tests, Minitab for Windows Rel 13 for correlation analysis and MS Excel for Windows for preparing the interaction graphs based on the mean values and their standard error (SE) according to Riley [14].

III. RESULTS AND DISCUSSION

Based on the p-value of the source of variation, the ANOVA results show that almost all the observation variables show significant interaction effects between biofertilizer inoculation and varieties of soybean tested, except for root volume and trifoliate number at harvest of the plant biomass (50 DAS). The treatment factor of biofertilizer inoculation also shows significant main effects on almost all the observation variables except for trifoliate number per clump at 14 DAS (TN-14) and plant height at 28 DAS (PH-28), while the differences between varieties are non-significant only in terms of trifoliate number per clump on 14 DAS, trifoliate number at biomass harvest and root volume (Table 1).

From the development of trifoliate number and plant height during the experiment, it appears that among the biofertilizer treatments, co-inoculation with both types of biofertilizer (B2 treatment) shows the fastest increase in both the average trifoliate number per clump (Fig. 1) and plant height (Fig. 2) compared with other treatments whereas among the varieties tested, Biosoy-2 variety (V2) shows the slowest increase in both the average trifoliate number per clump (Fig. 1) and plant height (Fig. 2) compared with other varieties of soybean. However, during the pod setting (42 DAS), on average, the treatment with *Rhizobium* inoculation (B1) shows the highest trifoliate number, while among the varieties, Dena-1 variety was the highest in trifoliate number at 42 DAS (Table 2). On the other hand, plant height was on average highest on soybean receiving co-inoculation with both types of biofertilizer (B2 treatment), while among the varieties tested, Detap and Dena-1 varieties show the highest plant height at pod setting stage (Table 2).

The results of measurement of the plant biomass samples (or destructive samples) are little bit different from the non-destructive plant samples, which show that there are no significant differences among the varieties tested in root volume and trifoliate number per clump at 50 DAS (Table 3). In addition, these variables did not show significant interaction between the treatment factors (Table 1). However, pod number and biomass weight per clump as a measure of potential yield, in addition to showing significant interaction between the treatment factors, are significantly different between varieties as well as between biofertilizer treatments (Table 1). The biomass weight per clump and root volume and nodule number per clump showed similar patterns of significant differences between the biofertilizer treatments, in which co-inoculation with both types of biofertilizer significantly increased biomass weight per clump (Table 3).

Table 1. The p-values of ANOVA results for all observation variables

Variables	Blocks	Biofertilizer inoculation	Varieties	Interaction
Trifoliate number at 14 DAS (TN-14)	0.0592	0.3556	0.1828	**0.0199**
Trifoliate number at 28 DAS (TN-28)	0.1171	**0.0018**	**0.0074**	**0.0033**
Trifoliate number at 42 DAS (TN-42)	0.7036	**0.0269**	**0.0020**	**0.0018**
Plant height at 14 DAS (PH-14)	0.8411	**0.0428**	**0.0000**	**0.0263**
Plant height at 28 DAS (PH-28)	0.7256	0.1626	**0.0000**	**0.0265**
Plant height at 42 DAS (PH-42)	0.1062	**0.0012**	**0.0000**	**0.0293**
Plant height at harvest (PHH)	**0.0080**	**0.0030**	**0.0000**	**0.0200**
Trifoliate number at harvest (TNH)	0.8871	**0.0138**	0.1062	0.1109
Root nodule number	**0.0025**	**0.0000**	**0.0222**	**0.0002**

Root volume	0.2249	**0.0000**	0.0569	0.3740
Pod number per clump	0.4170	**0.0000**	**0.0000**	**0.0006**
Dry biomass weight per clump	0.0751	**0.0000**	**0.0000**	**0.0000**

Table 2. Average trifoliate number per clump at 14 (TN-14), 28 (TN-28), and 42 DAS (TN-42), and plant height at 14 (PH-14), 28 (PH-28), and 42 DAS (PH-42) for each levels of the treatment factor

Treatments	TN-14 (number/clump)	TN-28 (number/clump)	TN-42 (number/clump)	PH-14 (cm)	PH-28 (cm)	PH-42 (cm)
B0: Control	3.83 a	7.65 b	16.36 ab	10.58 ab	18.54 a	30.86 b[1)]
B1: *Rhizobium*	4.04 a	8.29 a	17.43 a	10.94 a	19.82 a	34.78 a
B2: Rhizo+Myc	3.97 a	8.54 a	16.17 b	9.82 b	19.73 a	35.42 a
Tukey's HSD	ns	0.54	1.16	1.07	ns	2.76
V1: Detap	3.80 a	8.11 ab	16.77 ab	12.20 a	22.31 a	37.71 a
V2: Biosoy-2	4.09 a	7.81 b	15.63 b	10.00 b	16.14 c	25.64 b
V3: Dena-1	3.96 a	8.57 a	17.57 a	9.15 b	19.64 b	37.71 a
Tukey's HSD	ns	0.54	1.16	1.07	1.82	2.76

[1)] The same letters indicate non-significant different beween levels of a treatment factor based on Tukey's HSD test.

Table 3. Average trifoliate number per clump at biomass harvest (TNH), plant height at biomass harvest (PHH), nodule number per clump, root volume, pod number, and biomass weight per clump for each levels of the treatment factor

Treatments	TN-harvest (number/clump)	PH-harvest (cm)	Nodule (number/clump)	Root volume (mL)	Pod number per clump	Biomass weight (g/clump)
B0: Control	27.67 b	48.11 b	3.22 c	1.89 c	16.11 b	11.25 c[1)]
B1: *Rhizobium*	33.56 ab	52.50 ab	29.11 b	2.89 b	34.44 a	19.78 b
B2: Rhizo+Myc	38.33 a	55.67 a	40.78 a	3.94 a	37.78 a	23.28 a
Tukey's HSD	8.20	4.74	5.83	0.64	9.25	2.39
V1: Detap	32.44 a	57.44 a	22.44 b	2.67 a	15.22 c	15.22 b
V2: Biosoy-2	30.00 a	41.33 b	22.22 b	2.78 a	25.00 b	15.64 b
V3: Dena-1	37.11 a	57.50 a	28.44 a	3.28 a	48.11 a	23.45 a
Tukey's HSD	ns	4.74	5.83	ns	9.25	2.39

[1)] The same letters indicate non-significant different beween levels of a treatment factor based on Tukey's HSD test.

However, there were significant interaction effects of the treatment factors on biomass weight and pod number per clumps as well as all growth variables except root volume and trifoliate number at biomass harvest (Table 1). The patterns of interaction effects between the treatment factors are presented in Fig. 3 to Fig. 12, while trends in the increase of average trifoliate number per plant and plant height are presented in Fig. 1 and Fig. 2 respectively.

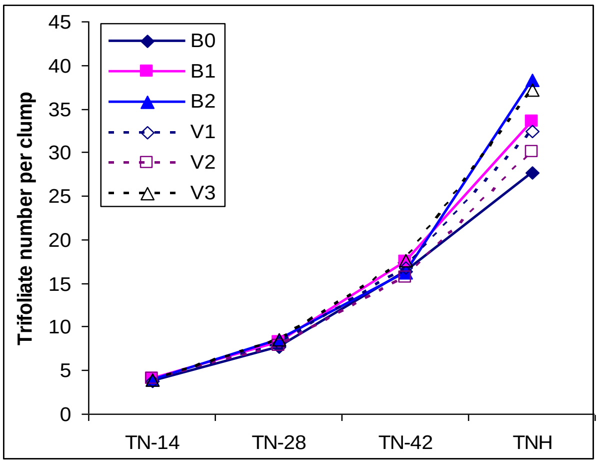

Fig.1. Development of trifoliate number of the soybean plants up to 50 DAS

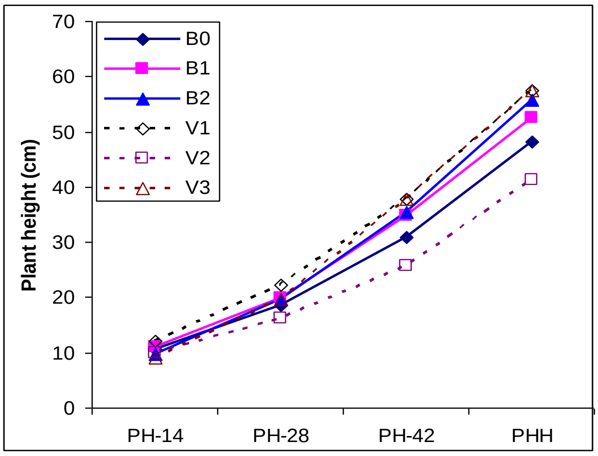

Fig.2. Development of the soybean plant height up to 50 DAS

Unlike growth trend of trifoliate number (Fig. 1), growth trend of plant height looks different between treatments and between varieties (Fig. 2). Based on the significant interaction effects, however, there seems to be differences of growth rate of plant height between varieties from the first measurement, i.e. 14 DAS (Fig. 3) to last measurement in the field, i.e. 42 DAS (Fig. 5). From the twice measurements, V1 seems to be the fastest in increasing their plant height both at 14 DAS and 28 DAS, but between the treatments it appears that the co-inoculation treatment resulted in fastest increase in plant height, especially in V1 (Fig. 1 to Fig. 2 to Fig. 3), but in V3, *Rhizobium* inoculation seems to result in faster increase from Fig. 3 to Fig. 4 to Fig. 5.

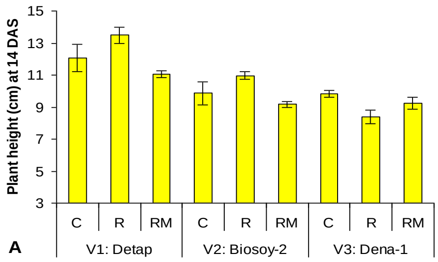

Fig.3. Plant height (Mean ± SE) at 14 DAS due to interaction between the treatment factors

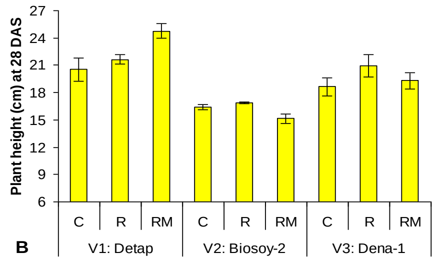

Fig.4. Plant height (Mean ± SE) at 28 DAS due to interaction between the treatment factors

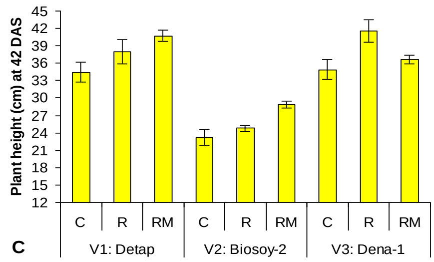

Fig.5. Plant height (Mean ± SE) at 42 DAS due to interaction between the treatment factors

Based on the interaction effect on pod number per clump (Fig. 9), there were different responses of different varieties of soybean to the treatments, in which V3 shows the highest pod number under co-inoculation but V2 shows the highest pod number under *Rhizobium* inoculation, while V1 shows no significant differences between co-inoculation and inoculation with *Rhizobium*, but soybean plant under control shows significantly lower pod number per clump (Fig. 9). These trends in pod number per clump between treatments (Fig. 9) were almost similar to the trends in biomass weight per clump between treatments (Fig. 10). These two variables produced the highest coefficient of correlation, with an R^2 = 77.26% (p-value <0.001) (Table 4), which statistically means that 77.26% of variation in pod number per clump is determined by variation in biomass weight per clump. The next strongest correlated variable to pod number was trifoliate number at

biomass harvest date (TNH) with an R^2 = 51.12% (p-value <0.001) (Table 4).

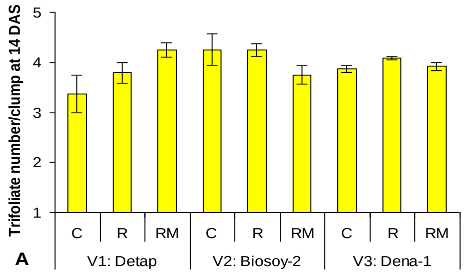

Fig.6. Trifoliate number per clump (Mean ± SE) at 14 DAS due to interaction between the treatment factors

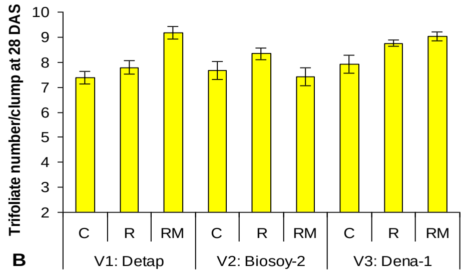

Fig.7. Trifoliate number per clump (Mean ± SE) at 28 DAS due to interaction between the treatment factors

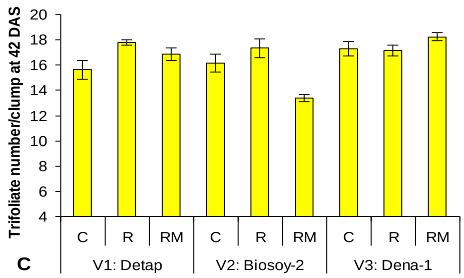

Fig.8. Trifoliate number per clump (Mean ± SE) at 42 DAS due to interaction between the treatment factors

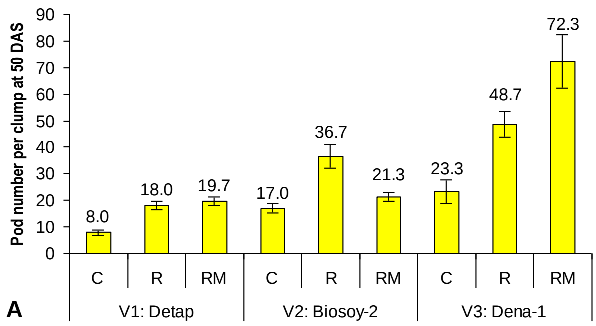

Fig.9. Pod number per clump (Mean ± SE) at 50 DAS due to interaction between the treatment factors

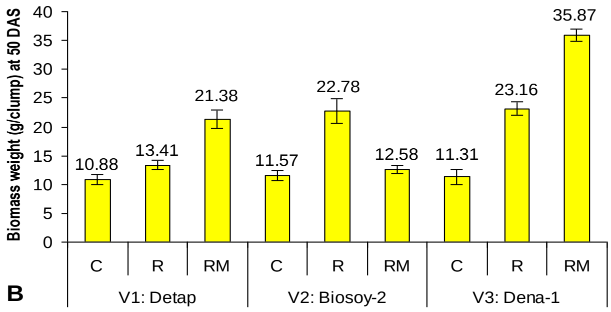

Fig.10. Biomass weight per clump (Mean ± SE) at 50 DAS due to interaction between the treatment factors

The next variables most closely correlated with pod and trifoliate number at harvest (TNH) were nodule number and root volume, and these two variables, as well as TNH and pod number, were highly correlated with trifoliate number at 28 DAS (TN-28). Therefore, it appears that high trifoliate number at 28 DAS was the most determining variable for high pod number per clump at the biomass harvest date. According experimental results by Portes et al. [15], until 17 days after emergence, photosynthate partition of soybean plants was mostly to roots, and soybean plants have a high capacity to partition their photosynthate to nodules for nodule maintenance and increasing N-fixing capacity.

Based on results in Table 4, nodule number was highly correlated with root volume, which means higher root volume is associated with higher nodule number. Kasperbauer et al. [16] also reported that soybean plants with larger root systems also produced more nodules. Since N_2 fixation by the *Rhizobium* bacteroids in soybean root nodules can produce up to 93% NH_4^+ [17], and it is available for N nutrition of the soybean plants, then the higher nodule number per clump would produce more N nutrient for soybean growth and yield formation.

According to the results reported by Collino et al. [18], shoot biomass of soybean in Argentina was highly correlated with N-fixation rates, with an R^2 = 0.520 or 52.0%. In this study, nodule number was also highly correlated with the biomass yield, with an R^2 = 60.37% (Table 4). In addition, pod number and biomass yield of soybean were significantly affected by inoculation, in which the highest mean values were in soybean plant receiving co-inoculation with *Rhizobium* and mycorrhiza biofertilizer (Table 3), although there were slight different responses to inoculation treatments between the soybean varieties tested, in which V2 showed lower pod number (Fig. 9) as well as lower biomass yield (Fig. 10) under co-inoculation with *Rhizobium* and mycorrhiza biofertilizer (B2) compared to inoculation with *Rhizobium* only (B1).

Table 4. Results of correlation analysis (correlation coefficient and p-value) between selected variables

Variables	TN-28	TN-42	PH-14	PH-28	PH-42	PH-h	TN-h	Nodules	Root vol.	Pods
TN-42	0.492									
p-value	**0.009**									
PH-14	-0.101	0.144								
p-value	0.617	0.473								
PH-28	0.528	0.409	0.439							
p-value	**0.005**	**0.034**	**0.022**							
PH-42	0.513	0.365	0.128	0.835						
p-value	**0.006**	0.061	0.525	**0.000**						
PH-harvest	0.746	0.546	0.137	0.821	0.866					
p-value	**0.000**	**0.003**	0.496	**0.000**	**0.000**					
TN-harvest	0.550	0.471	-0.158	0.272	0.347	0.531				
p-value	**0.003**	**0.013**	0.430	0.170	0.076	**0.004**				
Nodule number	0.561	0.239	-0.181	0.195	0.280	0.383	0.591			
p-value	**0.002**	0.230	0.365	0.330	0.158	**0.048**	**0.001**			
Root volume	0.484	0.108	-0.280	0.120	0.310	0.328	0.620	0.693		
p-value	**0.010**	0.591	0.158	0.550	0.115	0.095	**0.001**	**0.000**		
Pod number	0.618	0.421	-0.430	0.009	0.239	0.389	0.715	0.601	0.627	
p-value	**0.001**	**0.029**	**0.025**	0.964	0.230	**0.045**	**0.000**	**0.001**	**0.000**	
Biomass yield	0.763	0.508	-0.309	0.201	0.299	0.555	0.797	0.777	0.678	0.879
p-value	**0.000**	**0.007**	0.117	0.316	0.130	**0.003**	**0.000**	**0.000**	**0.000**	**0.000**

The higher pod number and biomass yield of soybean under co-inoculation with *Rhizobium* and mycorrhiza biofertilizer (B2 treatment), especially on soybean of V1 and V3 varieties, could be related to plant height at 50 DAS (Fig. 11) and nodule number per clump (Fig. 12), which were highest under co-inoculation treatment. These variables also significantly positively correlated with both pod number and biomass yield (Table 4). Egli [19] also reported that, on average, higher pod number per m^2 was associated with higher nod number per m^2.

In this study, taller soybean plants were associated with higher number of trifoliate, which also means higher nod number (Table 3 and Table 4). The higher nodule number in this study was highly associated with higher pod number, higher biomass yield, and higher trifoliate number (Table 4). The higher nodule number in soybean receiving co-inoculation with *Rhizobium* and mycorrhiza biofertilizer could be due to the positive contribution of the mycorrhiza biofertilizer, which mainly increases P uptake of the host plants, such as soybean plants [20].

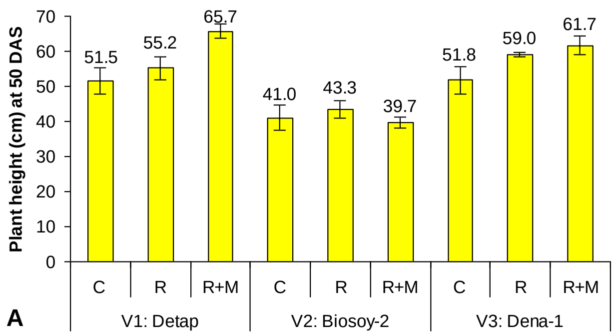

Fig.11. Plant height (Mean ± SE) at 50 DAS due to interaction between the treatment factors

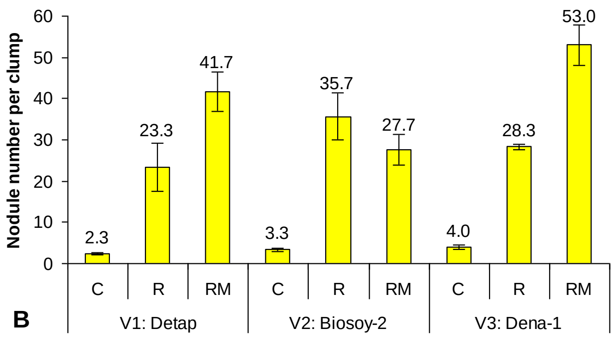

Fig.12. Nodule number per clump (Mean ± SE) at 50 DAS due to interaction between the treatment factors

According to the results reported by Miao et al. [21], increasing P supply in soybean increased both number and size of the nodules, and P deficiency reduced nodule development and N-fixation rates. Since soybean normally remobilizes N content of the shoot biomass for increasing growth of the developing seeds during the seed-filling stage [9], higher biomass yield accompanied with higher pod number would support for higher grain yield. By co-inoculation of soybean with *Rhizobium* and mycorrhiza biofertilizer, nodule number, pod number, trifoliate number and biomass yield became higher than in the other treatments. Igiehon and Babalola [22] also reported that *Rhizobium* and mycorrhizal fungi could increase soybean yield although under drought.

IV. CONCLUSION

It can be concluded that co-inoculation several varieties of soybean with *Rhizobium* and mycorrhiza biofertilizer increased yield potential of soybean in dryland as indicated by higher nodule number, trifoliate number, pod number and biomass yield of soybean, especially of Detap (V1) and Dena-1 (V3) varieties under co-inoculation treatment (B2), compared to inoculation with *Rhizobium* only (B1) or unioculated control (B0).

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